Supporting Information for

# CaMKII control of spine size and synaptic strength: role of phosphorylation states and nonenzymatic action

* To whom correspondence should be addressed: lisman@brandeis.edu

**This PDF file includes**

Figs. S1-S4

Supplementary methods

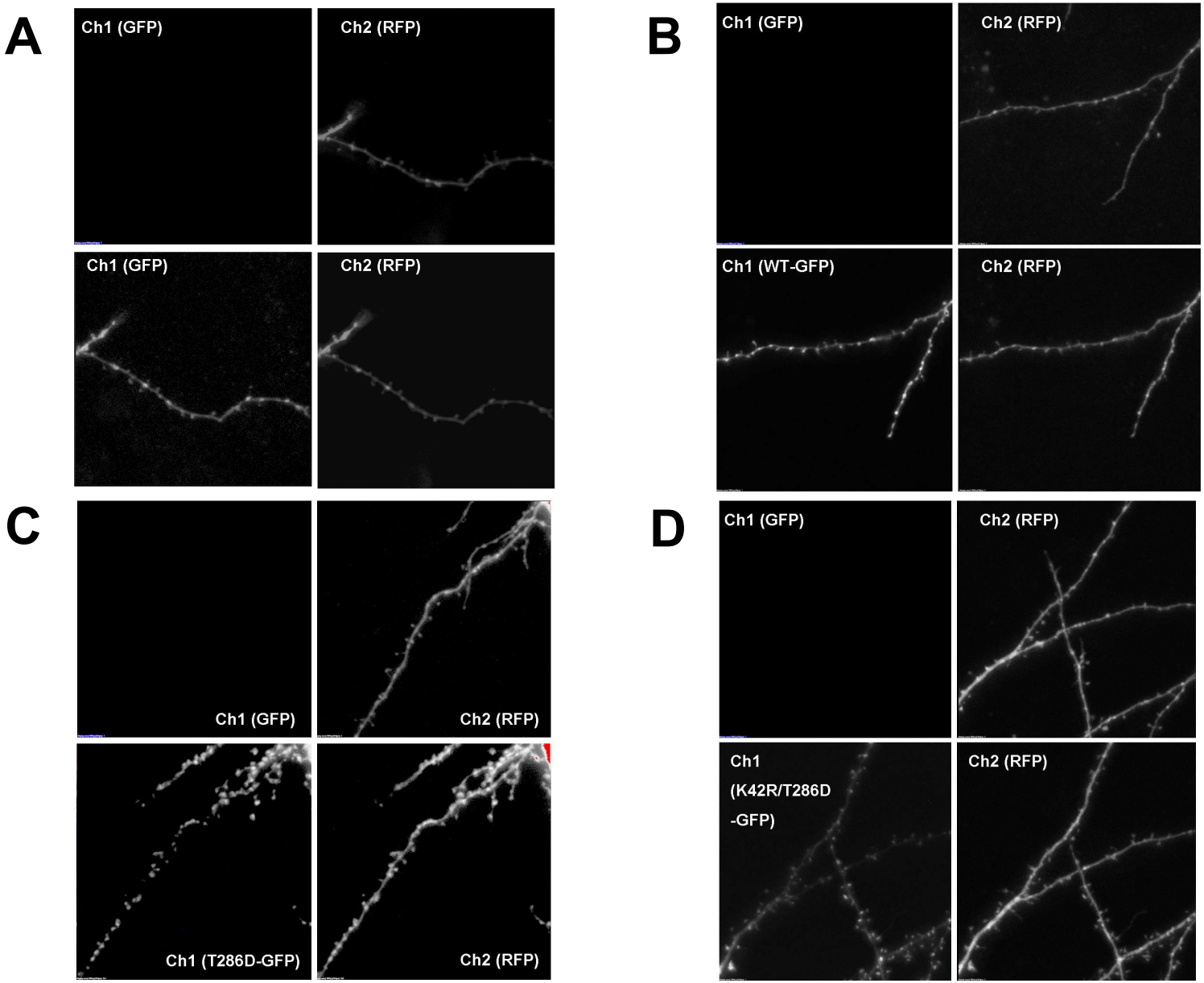


**Fig. S1.** Examples of confocal image data of DST. **A.** GFP. **B.** WT. **C.** T286D. **D.** K42R/T286D.

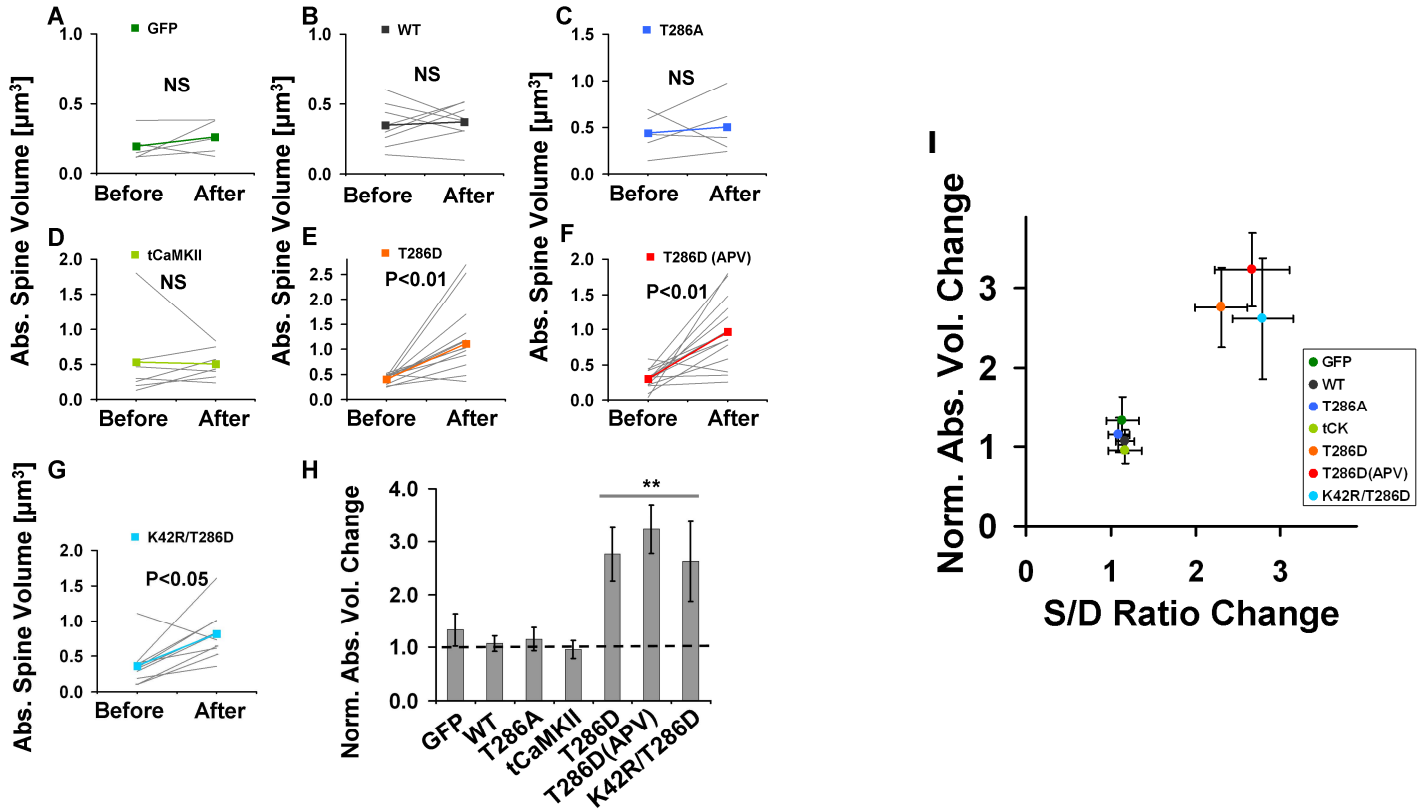


**Fig. S2.** Absolute volume analysis of DST data. **A.** eGFP: volume(before)= 0.19 +/-0.055 [µm$^3$], volume(after)= 0.26 +/-0.060; n= 5, p= 0.328. **B.** WT: volume(before)= 0.35 +/-0.060, volume(after)= 0.37 +/-0.051; n= 8, p= 0.709. **C.** T286A: volume(before)= 0.44 +/-0.069, volume(after)= 0.50 +/-0.095; n= 5, p= 0.653. **D.** tCaMKII: volume (before)= 0.53 +/-0.237 [µm$^3$], volume(after)= 0.51 +/-0.090; n= 7, p=0.893. **E.** T286D: volume (before)= 0.40 +/-0.026 [µm$^3$], volume(after)= 1.11 +/-0.202; n= 13, **p<0.01. **F.** T286D (APV): volume(before)= 0.30 +/-0.040 [µm$^3$], volume(after)= 0.96 +/-0.137; n=14, **p<0.01. **G.** K42R/T286D: volume(before)= 0.37 +/-0.095 [µm$^3$], volume(after)= 0.81 +/-0.115; n= 10, **p<0.01. **H.** Summary of normalized absolute volume changes. GFP: 1.33 +/- 0.307. WT: 1.06 +/- 0.148. T286A: 1.15 +/- 0.218. tCaMKII: 0.95 +/- 0.170. T286D: 2.76 +/- 0.504. T286D (APV): 2.66 +/- 0.441. K42R/T286D: 2.79 +/- 0.356. **I.** Correlation diagram between normalized absolute volume change and S/D ratio change.

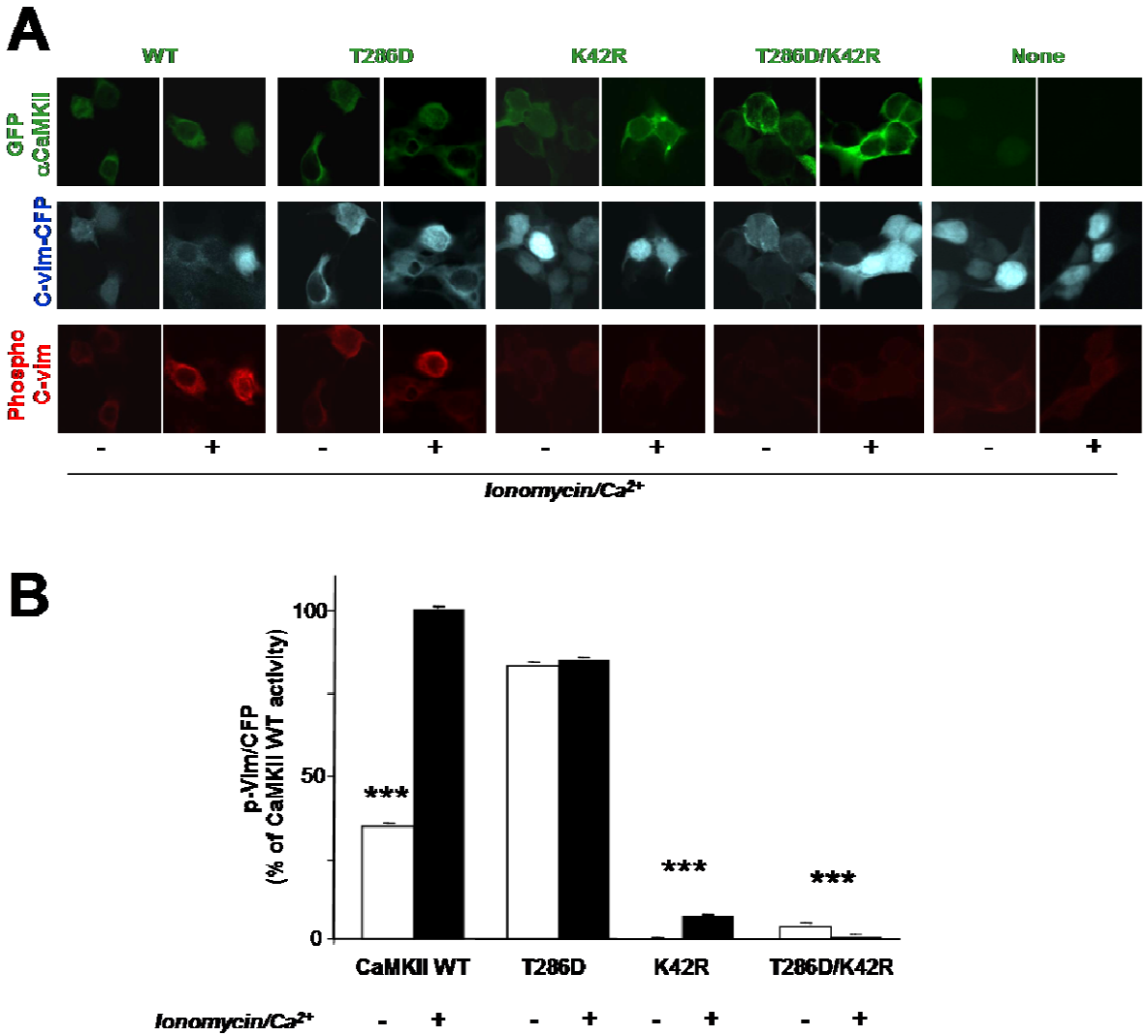


**Fig. S3.** Lack of phosphorylation activity of K42R and K42R/T286D mutation on αCaMKII. **A.** Photomicrographs of HEK cells co-transfected with CaMKII phosphorylation reporter (cytoplasmic-vim-CFP, or C-vim-CFP) and either WT, T286D, K42R or T286D/K42R-mGFP-αCaMKII, as indicated. Cells were stimulated or not with ionomycin/$Ca^{2+}$, as indicated. **B.** The quantification of mean (±SEM) C-vim phosphorylation reveals that K42R and K42R/T286D bear no detectable phosphorylation activity in HEK cells. n=9 (WT), 7 (T286D), 18 (K42R), 15 (K42R/T286D) *** Statistically different from CaMKII WT stimulated with ionomycin/$Ca^{2+}$, $p<0.001$, t-test.

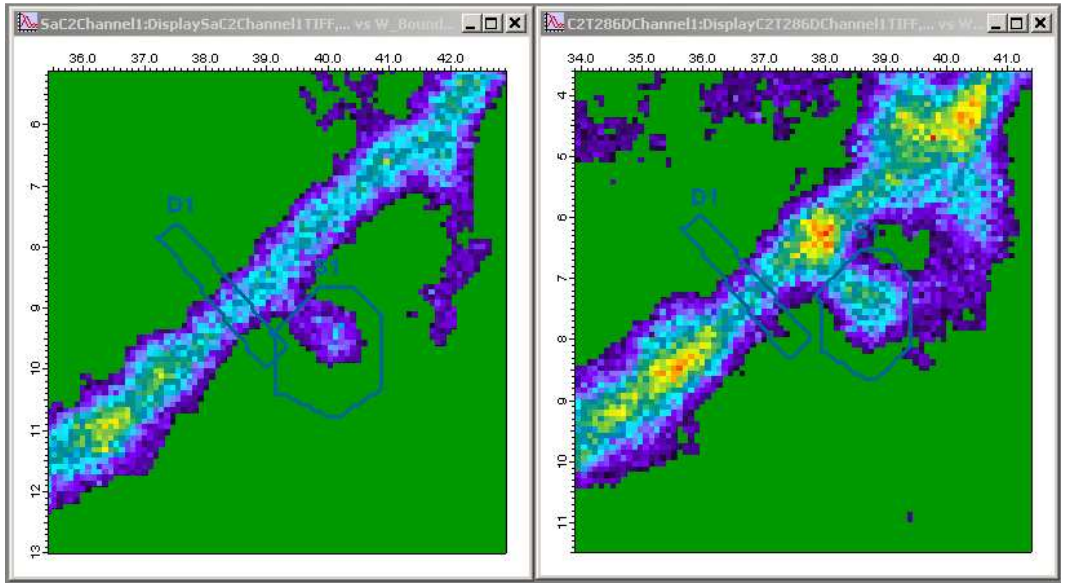


**Fig. S4.** The measurement of the relative spine volume (S/D ratio). Fluorescence of spines is normalized to that of dendritic segments in the same locations. From T286D experiments.

**Supplementary methods**

***Quantification of specific substrate (C-vim-CFP) phosphorylation by CaMKII.***

HEK cells were transfected with CaMKII phosphorylation reporter C-vim-CFP (developed by Tsui et al, 2005) together with various mGFP-αCaMKII constructs (WT, T286D, K42R, T286/K42R, none), using lipofectamine 2000 as described previously (Hudmon et al, 2005). On the next day, the cells were stimulated or not with ionomycin/$Ca^{2+}$ for 5 minutes and immediately fixed as described (Hudmon et al 2005). The degree of C-vim phosphorylation was assayed by immunostaining with monoclonal antibody MO82 (Tsui et al, 2005; 1:5000, MBL international Corporation), revealed with goat anti mouse Alexa 594, 1:1000, Invitrogen).

Images of mounted coverslips were taken on a Zeiss LSM510 confocal microscope with 63X, 1.4 NA oil immersion objective. Fluorescent signals were detected as follows: CFP: 458 laser line, emission filter BP480-520; GFP: 488 laser line, emission filter BP500-530; Alexa 594: 532 laser line, emission filter BP565-615). No correction was needed for the minor CFP-GFP bleed through.

Images were analysed with MetaMorph software. Phosphorylation levels were scored as Alexa594/CFP signals (after background subtraction) from regions of interest encompassing individual cells. Cells exhibiting similar levels of GFP signals were selected to minimize CaMKII expression variability. We also measured for any endogenous CaMKII activity present in HEK cells using cells transfected only with C-vim-CFP (see right images in Fig. S3A); the very low level of mean Alexa594/ CFP measured in those cells was subtracted from the measurements from all CaMKII transfected cells. Values were then normalized to that of the ionomycin/$Ca^{2+}$ WT-CaMKII condition (set at 100%).